Overview: A Rare Opportunity or Just One Less Reason to Be Depressed

A year ago, in this journal, Zhang and coworkers (Zhang et al., 2005) reported that the gene encoding the enzyme responsible for the synthesis of brain serotonin, tryptophan hydroxylase-2 (THP2), exhibited a functional variant associated with major (or unipolar) depression. The variant in question (G1463A) altered a critical amino acid (Arg441His) in the enzyme such that, at least in a transfected cell model, serotonin synthetic capacity was significantly attenuated. Even more striking, the frequency of the variant was increased in a cohort of depressed subjects, reinforcing a common suspicion that the availability of or response to serotonin is altered in depression. Although serotonin has been studied in the brain for decades, only in the past few years has the central role of TPH2 become clear. Like the sighting of a long-suspected but as yet uncharted island, the discovery of functional variation in human TPH2 associated with depression was heralded as a major opportunity to move from correlation to causality and anchor a flag for the serotonin hypothesis of depression on terra firma.

But the party was short lived. As noted by the letters that follow this overview, several groups with much larger samples simply haven't found the variant—at all—in their collections of depressed subjects, unipolar or bipolar. And not even in their controls. My own group also found an absence of the variant in our own depression cohort (H.C. Prasad, E. Sanders-Bush, R.C. Shelton, and R.D.B., unpublished data). In such circumstances, the possibility of an experimenter mistake looms large among possible explanations. However, by sharing their DNAs with several of the groups attempting replication, Caron and workers put this concern to rest. It is possible that replicates can fail simply because the sample sizes are too small. Here, however, we see nearly 5000 subjects sampled from multiple ethnic groups in the replicate attempts, making the findings of Zhang et al., as one correspondent infers, "a million to one shot." Given that the results have been validated by other groups using the Zhang et al. samples (a good practice for those of us analyzing rare mutations), the findings point to something quite distinct and possibly very important apart from depression per se in the subjects studied by Zhang et al. that enriches for the TPH2 Arg441His variant. The subjects studied by Zhang et al. are elderly, and many are severely depressed. Rare alleles in a number of genes, several now known to present with functional phenotypes (Halushka et al., 1999; Hahn et al. 2005), have been isolated by using blood pressure extremes to cull out contributors to hyper/hypotension. Perhaps the extremes of the depression spectrum have been sampled by Zhang et al., although this raises the question of how they could find it in their control samples (yet others do not see this either). A reasonable person could claim, today, that these variants track with something having nothing to do with depression, just a shared characteristic yet to be recognized and controlled for. While I doubt that this is likely to be the case, I admit that my view is only a hunch. The identification of multiple homozygotes carrying alleles that no one can find in single copy seems too low a probability not to point to a key depression trait enriched in the Zhang et al. samples. Clearly, we need to know more about the subjects of Zhang et al. Of course, having access to their brain serotonin levels would be helpful, though possibly CSF serotonin metabolites may be all that we can reasonably expect.

In their response to the correspondents below, Caron and colleagues suggest a key trait linked to the Arg441His substitution may be severe depression that is refractory to conventional medications, necessitating ECT. Presumably what led to the initial findings was a shared trait linked both to serotonin and depression, but not depression itself. It would be ideal to track these traits, or endophenotypes, within the families of the Zhang et al., cohort, though the elderly nature of the cohort leaves open the possibility that these traits emerge only late in life, a depressing thought considering the finite lifetime of the funding that sustains such studies. In time, the sampling of additional collections bearing greater similarity to that of Zhang et al. in search of Arg441His may clarify the issue. Until then, we must conclude that this TPH2 allele (as well as any other TPH2 coding variant, based on resequencing efforts described below) is not a major determinant of genetic risk for major depression. Other alleles, for example in the TPH2 promoter, may still be worth exploring, as evidence suggests that some form of variation at or near the TPH2 locus associates more broadly with major depression (Zill et al., 2004). The rarity of the Arg441His mutation doesn't mean that it is a less valuable finding—it may be a single nugget that points to a rich vein that we can mine for other gold. Recall just how few people have α synuclein or presenillin mutations, relative to the numbers stricken with Parkinson's and Alzheimer's disease, respectively. Yet the study of the pathways influenced by these genes are now seen as being central to efforts to treat and one day eradicate these disorders.

Under a common disease/rare variant framework, there may simply be no single gene variant imposing a major effect on population risk for depression. Given that more than 100 genes are likely to be involved in the birth, development, function, and plasticity of serotonergic neurons (there are more than a dozen serotonin receptors, and the serotonin transporter alone is now known to associate with more than half a dozen proteins), even a serotonin centrist should expect contributions from many points in the network for something as complex an endpoint as depression. Regardless, a flag has been planted, land claimed for the amine, land that was only suspected to be out there before. It is something of a brave new world for sure, and some

yet do not believe it truly exists. What manner of discovery Arg441His truly is, only time will tell. I, for one, would gamble on treasures beyond the beachhead. But it may be a treasure of small gems, one that must be appreciated in the context of other jewels, mined elsewhere and assembled patiently over time, to be a crowning achievement.

Randy D. Blakely[1,*]
[1] Department of Pharmacology and Psychiatry and Center for Molecular Neuroscience,
Vanderbilt University School of Medicine
Nashville, TN 37232-8548

*Correspondence: randy.blakely@vanderbilt.edu

Selected Reading

Hahn, M.K., Mazei-Robison, M.S., and Blakely, R.D. (2005). Mol. Pharmacol. *68*, 457–466.

Halushka, M.K., Fan, J.B., Bentley, K., Hsie, L., Shen, N., Weder, A., Cooper, R., Lipshutz, R., and Chakravarti, A. (1999). Nat. Genet. *22*, 239–247.

Zhang, X., Gainetdinov, R.R., Beaulieu, J.-M., Sotnikova, T.D., Burch, L.H., Williams, R.B., Schwartz, D.A., Krishnan, K.R.R., and Caron, M.G. (2005). Neuron *45*, 11–16.

Zill, P., Baghai, T.C., Zwanzger, P., Schule, C., Eser, D., Rupprecht, R., Moller, H.J., Bondy, B., and Ackenheil, M. (2004). Mol. Psychiatry *9*, 1030–1036.

DOI 10.1016/j.neuron.2005.11.029

Response to Zhang et al. (2005) Loss-of-Function Mutation in Tryptophan Hydroxylase-2 Identified in Unipolar Major Depression. Neuron *45*, 11–16

Zhang et al. reported a naturally occurring Arg441His missense variant of the human tryptophan hydroxylase-2 (*TPH2*) gene. The His441 allele was reported to be more abundant in a cohort of 87 depressed patients compared to 219 controls (Zhang et al., 2005). The frequency of His441 was higher in the depressed patients (0.06), among whom there were two His/His homozygotes and seven heterozygotes. His441 was also observed among the 219 controls (allele frequency 0.009), among whom one His/His homozygote and two Arg/His heterozygotes were detected. This reported association with depression is of note in the context of the effect of this substitution in reducing serotonin synthesis by approximately 80% in a heterologous expression assay in a rat cell line (Zhang et al., 2005) and through the observation of the role of *TPH2* variants as genetic predictors of depression (Zill et al., 2004) and response to antidepressants (Peters et al., 2004).

The authors of this letter represent three independent groups of investigators who have resequenced the relevant region of *TPH2* in some 779 unrelated individuals (Table 1), including 403 with major depression (ages 19–74, n = 21 > 60 years). In addition, another 1740 individuals with major depression (from the STAR*D study, ages 18–75, n = 121 > 60 years) were genotyped (Table 1). The sequenced and genotyped individuals represent five ethnic populations. Psychiatric assessment was accomplished using semistructured psychiatric interviews: NIAAA, SCID or SADS-L; NIMH and UCSF, SCID-I/P. Major depression was diagnosed by DSM-III-R or DSM-IV criteria, and a DSM-IV checklist was used for the STAR*D samples. Additional descriptions of individual data sets have been reported (Nielsen et al., 1998; Robin et al., 1997; Roy, 2003; Peters et al., 2004; Rush et al., 2004). All data were collected following informed consent and under human research protocols approved by IRBs of the respective institutions. For direct sequencing, genomic DNA was amplified by PCR with primers encompassing the Arg441His variant, sequenced using the BigDye Terminator V3.1 (Applied Biosystems Inc., Foster City, CA) and analyzed on ABI 3100 or 3730 sequencers. For genotyping, assays were performed using 5′-nuclease assay (TaqMan, ID # PMT06-55) and analyzed on an LJL plate reader (Molecular Devices, Sunnyvale, CA). 20% of the

Table 1. Individuals Sequenced or Genotyped for *TPH2* Arg441His

	Direct Sequencing				Genotyping
	NIAAA	UCSF	NIMH	Total	NIMH
Major depression	**295**	**108**		**403**	**1740**
Caucasian	124	83		207	1411
African American	63	8		71	213
American Indian	101	0		101	0
Hispanic	0	11		11	0
Asian-American	2	6		8	0
Others	5	0		5	116
Control	**152**	**200**		**352**	
Caucasian	87	100		187	
African American	63	100		163	
American Indian	2	0		2	
Bipolar			**24**	**24**	
Caucasian			24	24	
African American					
American Indian					
Total	**447**	**308**	**24**	**779**	**1740**
Grand total					**2519**